RIDA® Aviditätsreagenz

Art. No.: LB0023

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1. Intended use

For *in vitro* diagnostic use. The RIDA® Aviditätsreagenz is an additional reagent to the RIDA®LINE Parvovirus B19 IgG blot for determination of IgG avidity.

2. General

The classical serology of infectious diseases is based on the observation that IgM-antibodies specific to a certain type of pathogen are formed only temporarily, whereas the respective IgG response continues for a long time. For this reason, IgM diagnosis indicates acute infection, but IgG diagnosis without parallel IgM is a sign of past infection. Due to the variability of immune response and to the occurrence of aberrant serological processes (e.g. persistent, reactivated or absent IgM response) this classical approach may lead to false conclusions in many cases.
Avidity describes the binding strength of specific antibody to antigen. It was found to be low in the first phase after primary infection but then to increase over time. In addition to classic serodiagnosis, measurement of avidity provides information making it possible to distinguish between acute and past infection. During IgG response there is a continuous increase in the avidity of IgG for the respective antigen. This occurs as an immunological principle. For this reason, IgG with a low avidity is usually present in acute infection and IgG with high avidity is present after infection has ended. As a result of this observation, another very significant marker has been introduced into diagnosis in the last few years: the avidity of IgG.

3. Test principle

Two test strips are incubated with the diluted serum sample for each test run. During this initial incubation, antibodies in the sample bind to their specific antigens, which are fixed on the test strips. Then one of the two test strips is washed with avidity solution. During this step, the low avid antibodies are removed by diffusion while the high avid antibodies remain bound to their specific antibodies. Following the washing step, anti-human antibodies conjugated with horse radish peroxidase are added. The specific antibodies bound to their antigens is made visible by adding a substrate that forms bands on the test strips. The relative position of the coloured bands indicates the specificity of the reacting antibodies. Subsequent comparison of the two test strips then provides a basis for measuring the avidity of the antibodies and for establishing the stage of infection.

4. Reagents provided

The reagents are sufficient for 25 determinations. Each pack contains a bottle with:

Avidity	25 determ.	Avidity reagent (solid); for approx. 60 ml ready-to-use solution

5. Storage instructions

The expiry date of the unopened avidity reagent (solid) is as indicated on the label. The reagent can be stored in the refrigerator or at room temperature (2 oppure entro 5 giorni se conservati a temperatura ambiente (20 – 25 ℃). In connection with the RIDA®LINE Parvovirus B19 IgG, the expiry date of the LINE blot must not be exceeded. The avidity reagent (solid) must be protected from moisture.
The ready-to-use solution can be stored in the refrigerator at 2 - 8 ℃ for 8 weeks. It may be stored at -20 ℃ over a period of 12 mont hs. In this case, the avidity solution should be thawed in warm water (for about 45 minutes).

6. Materials required but not provided

6.1. Reagents

– RIDA®LINE Parvovirus B19 IgG (Art. No. LB6023)

6.2. Accessories

– Measuring cylinder for 50 ml

7. Warnings and precautions for the users

For *in vitro* diagnostic use only.

The test has to be used only by experienced laboratory staff. Please refer to guidelines for safety regulations in medical laboratories. The test protocol must be followed strictly.

When handling the reagent (solid), equipment designed to protect the respiratory system and eyes must be used. Use of clothing designed to protect the skin is also recommended. The ready-to-use solution can be used without respiratory protection. Do not mouth pipette the reagent. Avoid contact with skin or mucous membranes. After contact, rinse off/out with plenty of water.

8. Preparation of the ready-to-use avidity solution

The weighed-out avidity reagent is dissolved in 40 ml of the ready-to-use wash / dilution buffer of the RIDA®LINE Parvovirus B19 test kit. This dissolving process takes some time and can be accelerated by mild warming. The solution has a final volume of about 60 ml.

9. Test procedure

9.1. Preliminary comments

Bring all kit components (RIDA®LINE Parvovirus B19 IgG and RIDA® Aviditätsreagenz) to room temperature (18 - 25 °C) prior to use for at least 30 minutes. Beside the specifications given here, all instructions of the RIDA®LINE Parvovirus B19 IgG insert are effective.
Two blot strips have to be used for each sample with every test run. To make a comparative evaluation possible, one of the two strips is treated with the avidity solution and the other not. The reproducibility of the results depends mainly on constant washing of the strips. The washing frequencies as mentioned above should therefore always be complied with.

9.2. First incubation

Two wells of the incubation tray are required for each sample. 2 ml of the ready-to-use wash / dilution buffer are pipetted into each well. A test strip is then carefully placed in each of the wells filled with wash buffer using a forceps.

The strips must be completely wetted with buffer. The strip numeration must show upwards.

20 µl each of the undiluted sample (serum or plasma) are added to the two corresponding wells (dilution 1:101). Please be sure to add the sample at one end of the immersed strips into the wash buffer and mix as soon as possible by shaking the tray carefully. During the incubation, the trays should be covered up with the enclosed lids.
The blot strips with the samples are placed on a horizontal shaker and are incubated for 1 hour at room temperature (18 - 25 °C).

9.3. Washing

Remove the solution (via suction), preferably with an attached disinfection trap. Then place 2 ml of the ready-to-use wash / dilution buffer in each well and wash on the shaker while shaking gently for 5 minutes at room temperature (18 - 25 °C). The wash buffer is aspirated after the washing procedure.

9.4. Second incubation

Add 2 ml of the wash / dilution buffer to one strip of the double determination and 2 ml of the ready-to-use avidity reagent to the other. Incubate the strips for **3 minutes** on a horizontal shaker at room temperature (18 – 25 °C). In this step, the low-avidity antibodies are "washed off". The corresponding second charge, which is not to be treated with the avidity solution, must be tested in parallel to each sample.

Important!
It is very important to incubate for <u>exactly three minutes</u>.

9.5. Washing

Remove the solution (via suction), preferably with an attached disinfection trap. Then place 2 ml of the ready-to-use wash / dilution buffer in each well and wash on the shaker while shaking gently for 5 minutes at room temperature (18 - 25 °C). The wash buffer is aspirated after the washing procedure.

9.6. Third incubation

Add 2 ml of the freshly prepared IgG conjugate solution to each strip. Incubate the strips for 45 minutes on a horizontal shaker at room temperature (18 - 25 °C).

9.7. Washing

Remove the solution (via suction), preferably with an attached disinfection trap. Then place 2 ml of the ready-to-use wash / dilution buffer in each well and wash on the shaker while shaking gently for 5 minutes at room temperature (18 - 25 °C). The wash buffer is aspirated after the washing procedure. Carry out this washing step a total of three times.

9.8. Fourth incubation

Add 1.5 ml of the ready to use Substrate solution to each strip. Incubate the strips for 5 - 10 minutes at room temperature (18 - 25 °C) , until the cut off band becomes visible. Thereafter, the substrate is removed via suction. Still in their wells, the strips should be rinsed shortly three times with deionized water to stop the color reaction.
The strips are carefully taken out of the wells with tweezers. To dry the strips, they are placed between filter paper for about 2 hours. After drying the strips can be adhesively attached to the evaluation sheet and the results can be recorded.

10. Evaluation

Compare the intensities of the corresponding bands on the two test strips incubated with the same serum. Verify if the intensities have changed.
The evaluation of the avidity strips has to be based on the criteria given in the insert of the RIDA®LINE Parvovirus B19 IgG test.
An antibody is considered to have low avidity if the intensity of the corresponding bands is reduced by at least 50 % in the avidity test. A complete reduction of all intensities is a relatively reliable sign of a fresh infection.
Generally, no absolute rules can be set up for avidity evaluation. The interpretation of avidity has to be done always within the context of the overall test results.

11. Limitations of the method

Deviations from the test procedure as described may falsify the interpretation of the results. It is absolutely necessary to keep incubation times as indicated.
All test results should be considered as supplements to the overall clinical picture. To confirm the diagnosis, the clinical findings and relevant medical history have to be considered as well.